

CLAIMS

1. A method of increasing carbon flow into a metabolic pathway of a PTS⁻/Glu⁻ bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising,
 - a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS⁻/Glu⁻ host cell by transforming the PTS⁻/Glu⁻ host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein;
 - b) allowing integration of the DNA construct to restore a Glu⁺ phenotype; and
 - c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a metabolic pathway of the transformed host cell is increased compared to the carbon flow into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions.
2. The method according to claim 1, wherein the promoter is a non-host cell promoter.
3. The method according to claim 1, wherein the promoter is a modified endogenous promoter.
4. The method according to claim 1, wherein the glucose assimilation protein is a glucose transporter.
5. The method according to claim 4, wherein the glucose transporter is a galactose permease obtained from *E. coli* or a glucose transporter having at least 80% sequence identity thereto.
6. The method according to claim 1, wherein the glucose assimilation protein is a phosphorylating protein.
7. The method according to claim 6, wherein the phosphorylating protein is a glucokinase.

8. The method according to claim 5, further comprising modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the $\text{PTS}^-/\text{Glu}^-$ host cell by transforming the
5 $\text{PTS}^-/\text{Glu}^-$ host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase.

9. The method of claim 1, wherein the bacterial host cell is selected from the group consisting of *E. coli* cells, *Bacillus* cells and *Pantoea* cells.

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10. The method according to claim 1, wherein the $\text{PTS}^-/\text{Glu}^-$ host cell is obtained from a PTS cell by deletion of one or more genes selected from the group consisting of *ptsI*, *ptsH* and *crr*.

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11. The method according to claim 1, further comprising transforming the $\text{PTS}^-/\text{Glu}^+$ host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase, and a phosphoenolpyruvate synthase.

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12. The method according to claim 1, further comprising transforming the $\text{PTS}^-/\text{Glu}^+$ host cell with a polynucleotide encoding at least one enzyme selected from the group consisting of DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase EPSP synthase and chorismate synthase.

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13. The transformed bacterial cell obtained according to the method of claim 1.

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14. A method for increasing the production of a desired product in a $\text{PTS}^-/\text{Glu}^-$ bacterial host cell originally capable of utilizing a PTS for carbohydrate transport comprising,

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a) transforming a bacterial host cell having a $\text{PTS}^-/\text{Glu}^-$ phenotype with a DNA construct comprising a promoter, wherein said DNA construct is chromosomally integrated into the $\text{PTS}^-/\text{Glu}^-$ host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein;

b) culturing the transformed bacterial host cell under suitable conditions;

c) allowing expression of the glucose assimilation protein to obtain a host cell having a $\text{PTS}^-/\text{Glu}^+$ phenotype; and

5 d) obtaining an increased amount of a desired product in the transformed bacterial host cell compared to the amount of the desired product produced in a corresponding PTS bacterial cell cultured under essentially the same culture conditions,

wherein said desired product is selected from the group consisting of pyruvate, PEP,
10 lactate, acetate, glycerol, ethanol, succinate and chorismate.

15 15. The method according to claim 14, wherein the host cell is selected from the group consisting of *E. coli* cells, *Bacillus* cells and *Pantoea* cells.

16. The method according to claim 14, wherein the glucose assimilation
15 protein is a galactose permease obtained from *E. coli* or a glucose transporter having at least 80% sequence identity thereto.

17. The method according to claim 14, wherein the glucose assimilation
20 protein is a glucokinase obtained from *E. coli* or a glucokinase having at least 70% sequence identity thereto.

18. The method according to claim 14, wherein the desired product is
25 chorismate.

19. The method according to claim 14, wherein the desired product is
succinate.

20. The method according to claim 14, wherein the desired product is
30 ethanol.

21. The method according to claim 14, wherein the desired product is
glycerol.

22. A method of increasing carbon flow into a metabolic pathway of a $\text{PTS}^-/\text{Glu}^-$ bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising,

5 a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a $\text{PTS}^-/\text{Glu}^-$ host cell by transforming the $\text{PTS}^-/\text{Glu}^-$ host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease;

10 b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the $\text{PTS}^-/\text{Glu}^-$ host cell by transforming the $\text{PTS}^-/\text{Glu}^-$ host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase;

15 c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an increase
20 in carbon flow into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in a corresponding unaltered $\text{PTS}^-/\text{Glu}^-$ bacterial cell.

23. The method according to claim 22, wherein the bacterial host cell is
25 selected from the group consisting of *E. coli* cells, *Bacillus* cells and *Pantoea* cells.

24. The method according to claim 22, wherein the metabolic pathway is the common aromatic pathway.

30 25. The method according to claim 22, further comprising transforming the $\text{PTS}^-/\text{Glu}^-$ host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase and a phosphoenolpyruvate synthase.

26. A method of restoring a Glu⁺ phenotype to a PTS⁻/Glu⁻ bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising

a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose transporter in a PTS⁻/Glu⁻ host cell by transforming the PTS⁻/Glu⁻ host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose transporter;

b) allowing integration of the first DNA construct, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucose transporter; and

c) allowing expression of the glucose transporter, wherein said expression restores a Glu⁺ phenotype to the PTS⁻/Glu⁻ host cell.

27. The method according to claim 26, wherein the host cell is selected from the group consisting of *E. coli* cells, *Bacillus* cells and *Pantoea* cells.

28. The method according to claim 26 further comprising modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS⁻/Glu⁻ host cell by transforming the PTS⁻/Glu⁻ host cell with a second DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase;

allowing integration of the second DNA construct wherein the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase; and allowing expression of the glucokinase.

29. The method according to claim 28, wherein the host cell is selected from the group consisting of *E. coli* cells, *Bacillus* cells and *Pantoea* cells.

30. The method according to claim 26, wherein the restored Glu⁺ cells have a specific growth rate of at least about 0.4 hr⁻¹.

31. The method according to claim 26, wherein the glucose transporter is a galactose permease.

32. A bacterial strain having the restored Glu⁺ phenotype obtained according to the method of claim 26.

33. A bacterial strain having the restored Glu⁺ phenotype obtained according
5 to the method of claim 28.

34. A method of increasing phosphoenolpyruvate (PEP) availability in a bacterial host cell comprising,

- 10 a) selecting a bacterial host cell having a PTS⁻/Glu⁻ phenotype, wherein the bacterial host was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport;
- b) modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct comprising a promoter, wherein said
15 DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein;
- c) culturing the transformed bacterial host cell under suitable conditions; and
- 20 d) allowing expression of the glucose assimilation protein to obtain an altered host cell having a PTS⁻/Glu⁺ phenotype, wherein the PEP availability is increased compared to the PEP availability in a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

25 35. The method according to claim 34, wherein the glucose assimilation protein is a galactose permease and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of the galactose permease.

30 36. The method according to claim 34, wherein the glucose assimilation protein is a glucokinase and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of a glucokinase.

35 37. The method according to claim 35 further comprising modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct

comprising a promoter, wherein said DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucokinase.

5 38. The method according to claim 34, wherein the bacterial host cell is an *E. coli* cell, a *Bacillus* cell or a *Pantoea* cell.

 39. The method according to claim 34 further comprising transforming the selected bacterial host cell with a nucleic acid encoding a transketolase, a
10 transaldolase or a phosphoenolpyruvate synthase.

 40. The altered host cell obtained according to the method of claim 34.

 41. A method for increasing the growth rate of a $\text{PTS}^-/\text{Glu}^-$ bacterial host
15 cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising,

 a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a $\text{PTS}^-/\text{Glu}^-$ host cell by transforming the $\text{PTS}^-/\text{Glu}^-$ host cell with a first DNA
20 construct comprising an exogenous promoter and DNA flanking sequences corresponding to (5') upstream region of the galactose permease;

 b) modifying an endogenous regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the $\text{PTS}^-/\text{Glu}^-$ host cell by transforming the $\text{PTS}^-/\text{Glu}^-$ host cell with a second DNA construct
25 comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase;

 c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces the endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct
30 replaces the endogenous promoter of the nucleic acid encoding the glucokinase

 d) culturing the transformed bacterial host cell under suitable conditions; and

 e) allowing expression of the galactose permease and the
35 glucokinase from the modified regulatory regions to obtain an altered bacterial cell having an increase specific growth rate compared to the specific growth

rate of a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

42. The altered bacterial cell obtained according to the method of claim 41.

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43. The altered bacterial cell of claim 41, wherein said bacterial cell is a *Pantoea* cell.

44. The altered bacterial cell of claim 41, wherein said bacterial cell is an *E. coli* cell.

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45. The method according to claim 41 further comprising transforming the selected bacterial host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase and a phosphoenolpyruvate synthase.

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46. A method for increasing the production of a desired product in a $\text{PTS}^-/\text{Glu}^-$ *E. coli* host cell originally capable of utilizing a PTS for carbohydrate transport comprising,

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a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in an *E. coli* $\text{PTS}^-/\text{Glu}^-$ cell by transforming the *E. coli* $\text{PTS}^-/\text{Glu}^-$ cell with a first DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease;

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b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the *E. coli* $\text{PTS}^-/\text{Glu}^-$ cell by transforming the *E. coli* $\text{PTS}^-/\text{Glu}^-$ cell with a second DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase;

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c) culturing the transformed *E. coli* $\text{PTS}^-/\text{Glu}^-$ cell under suitable conditions to allow expression of the galactose permease and expression of the glucokinase; and

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d) obtaining an increased amount of a desired product in the transformed *E. coli* cells compared to the amount of the desired product in a corresponding $\text{PTS}^-/\text{Glu}^-$ *E. coli* cell cultured under essentially the same

culture conditions wherein the desired product is ethanol, chorismate or succinate.

47. The method according to claim 46, wherein the exogenous promoter is a
5 non-native promoter selected from the group consisting of *Gl*, *trc*, *lac* and derivative promoters thereof.

48. The *E. coli* cells obtained according to the method of claim 46.